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Emergency Department point-of-care antiviral host response testing is accurate during periods of multiple respiratory virus co-circulation

Running title: Host response point-of-care testing for viruses

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Abstract

Objectives

FebriDx is a CE-marked, FDA-approved point-of-care test that detects the antiviral host response protein Myxovirus Resistance Protein A (MxA), in addition to C-reactive protein, using finger-prick blood. FebriDx MxA detection had a high negative predictive value for COVID-19 in symptomatic adults presenting to hospital in the first waves of the pandemic and was used subsequently as a 'rule out' triage tool in Emergency departments. The diagnostic accuracy of FebriDx MxA in the current context of co-circulation of influenza, SARS-CoV-2, and Respiratory Syncytial Virus (RSV), and in the era of COVID-19 vaccination, is unknown.

Methods

We retrospectively evaluated the diagnostic performance of FebriDx MxA in adults with acute respiratory symptoms presenting to the Emergency Department (ED) of a large UK teaching hospital using Reverse Transcription Polymerase Chain Reaction (RT-PCR) as the reference standard (Cepheid Xpert Xpress SARS-CoV-2/Flu/RSV).

Results

Between March 9th 2022 and March 8th 2023, 5426 patients had both FebriDx and RT-PCR testing with valid results. 999 (18.4%) of patients had influenza detected, 520 (9.6%) SARS-CoV-2, and 190 (3.5%) RSV. Negative Predictive Value (NPV) of MxA detection by FebriDx was 97.5% (96.9-98.0) for influenza, 97.1% (96.4-97.7) for SARS-CoV-2, 98.1% (97.5-98.6) for RSV, and 92.8% (91.8-93.7) for all viruses combined.

Conclusions

In symptomatic adults FebriDx MxA had a high NPV for influenza and RSV, and retained a high NPV for SARS-CoV-2, in the context of virus co-circulation and widespread COVID-19 vaccination. FebriDx continues to be a useful 'rule out' triage tool in the ED and could potentially be scaled to provide a national triage solution for future viral pandemics.

Keywords

Host response, point-of-care test, FebriDx, influenza, SARS-CoV-2, RSV.

Introduction

FebriDx (Lumos Diagnostics, USA) is a CE marked, FDA approved, lateral flow, point-of-care test that detects the antiviral host response protein Myxovirus Resistance Protein A (MxA) and C-reactive protein (CRP), from a finger-prick blood sample and generates a result in around 10 minutes. It was initially designed to distinguish bacterial from viral respiratory infections to assist in antibiotic decision making.[1] In the first wave of the COVID-19 pandemic, studies demonstrated that the detection of MxA using FebriDx testing had a high sensitivity and negative predictive value (NPV) for the detection of COVID-19 compared to the reference standard of Reverse Transcription Polymerase Chain Reaction (RT-PCR) testing, in adults presenting to hospital with acute respiratory illness.[2,3] In subsequent waves, it has been demonstrated that FebriDx testing markedly improved the triage of patients with suspected COVID-19 in the Emergency Department compared to clinical triage, and reduced the time that patients who did not have COVID-19 spent in a high-risk areas nursed alongside SARS-CoV-2–positive patients.[4] Several other studies and a meta-analysis have confirmed the high sensitivity and NPV of FebriDx MxA for COVID-19 in symptomatic patients being admitted to hospital.[5–7]

Diagnostic accuracy studies evaluating FebriDx MxA have predominantly been performed during the first or second waves of the pandemic, during which time contemporaneous circulation of other respiratory viruses was dramatically reduced.[8–10] Influenza, Respiratory Syncytial Virus (RSV), and other respiratory viruses associated with acute respiratory illness are now co-circulating with SARS-CoV-2 and also trigger an antiviral host response and may therefore be identified through FebriDx MxA testing.[11,12] Emergency Departments are often overcrowded and have been shown to be important sources of nosocomial infection with respiratory viruses.[13] Detection of influenza and RSV in addition to SARS-CoV-2 in patient presenting to ED would be clinically useful to identify infected patient early, segregate them, and prevent transmission to other patients in these areas. However, the accuracy of FebriDx MxA in influenza or RSV infection, and the impact of co-circulating respiratory viruses upon diagnostic accuracy for COVID-19, are unknown. In addition, most diagnostic accuracy studies of the FebriDx were performed before COVID-19 vaccination had become available. Over 75%

of people aged 18 years and over in England had received three COVID-19 vaccines by March 2023, with over 92% of people aged over 70 years old having received a fourth dose.[14] The impact of widespread vaccination on the accuracy of this host immune response diagnostic test is also unknown.

Therefore, we aimed to evaluate the diagnostic performance, and in particular the NPV, of FebriDx MxA for the detection of influenza, SARS-CoV-2, RSV, and all three viruses combined, in symptomatic adults in the Emergency Department to determine if it remains a valuable 'rule out' triage tool in the context of respiratory virus co-circulation and widespread SARS-CoV-2 vaccination.

Methods

We did a single-centre, retrospective, observational study in the Emergency Department (ED) in Southampton General Hospital, which is part of University Hospital Southampton NHS Foundation Trust, in Southampton, UK. All adult patients (aged 18 years and over) who presented to the ED with symptoms of cough, shortness of breath, unexplained fever, contact with known COVID-19 cases, or other clinical suspicion of COVID-19 were triaged to a COVID-19 high-risk cohort area with enhanced infection control measures. FebriDx testing was implemented as a standard-of-care point-of-care triage test in this high-risk cohort area in late 2020 following local validation and was performed by trained healthcare assistants. Patients with FebriDx MxA detected were classified as high risk for COVID-19 and remained in the high-risk cohort area. Patients who did not have MxA detected were reclassified as lower-risk and moved to lower-risk areas within the ED. FebriDx test results were uploaded to the OnBase electronic system (Hyland, USA) including a photograph of the completed test. Patients who were immunosuppressed or had been unwell for over 14 days were not tested by FebriDx. The CRP component of the FebriDx was not used for clinical decision making and so is not evaluated here.

We used routinely collected, anonymised clinical data including demographics (sex, ethnicity, age at attendance), inpatient admission status, length of hospital stay, in-hospital mortality, comorbidities, date of ED attendance, and time, date and results of FebriDx and RT-PCR tests.

We compared the diagnostic accuracy of the FebriDx MxA to the reference standard of RT-PCR using the Xpert Xpress SARS-CoV-2/Flu/RSV and Xpert Xpress CoV-2/Flu/RSV *plus* RT-PCR tests (Cepheid, USA) which are highly accurate diagnostic assays that detect Influenza A and B, RSV and SARS-CoV-2.[15–18] These assays were used as a near patient test in our ED, and our Acute Medicine Unit, and were introduced in March 2022. Patients were tested by RT-PCR if there was felt to be a high likelihood of admission to hospital from the ED. We combined influenza A and B test results for the purposes of this study.

We included all patients with both a valid FebriDx test and RT-PCR test result performed within 24 hours of each other.

Patients classified as having a low level of viral RNA detected for viruses (Ct Value ≥35) were excluded from this evaluation as the clinical pathway for these patients differed from those

with standard levels of viral RNA detected (Ct <35). Patients with one respiratory virus detected at a low level of RNA but another virus detected (Ct <35) remain included, with the virus with a low level of RNA detected classified as not detected.

The study was approved as a clinical service evaluation with University Hospital Southampton NHS Foundation Trust (UHS reference: 7409), and the Trust's data protection team. The study was reviewed by UHS Research and Development governance team who confirmed that regional ethics committee review was not required.

There was no specific funding for this study and all the FebriDx and RT-PCR tests were purchased by University Hospital Southampton NHS Foundation Trust as part of providing routine clinical service. The manufacturers of the FebriDx and RT-PCR tests had no input into the trial design, conduct, analysis, or the writing of the manuscript. This report conforms to the STARD reporting guidelines for diagnostic accuracy studies.[19]

Statistical analysis

Baseline characteristics were summarised for all patients. Baseline characteristics for patients with influenza, SARS-CoV-2, and RSV detected by RT-PCR were also summarised, and selected characteristics between these groups were compared using the Kruskal-Wallis test for continuous data and chi-squared for categorical data. Measures of diagnostic accuracy (sensitivity, specificity, positive predictive value, negative predictive value) of FebriDx MxA were calculated along with 95% confidence intervals (95%CI), compared to the reference standard of RT-PCR for influenza, RSV, SARS-CoV-2 individually, and for all three viruses combined. Diagnostic accuracy of FebriDx MxA was also calculated separately for one eight-week period of high SARS-CoV-2 detection by RT-PCR when influenza and RSV detections were low ('Period 1'), and for one eight-week period of high detection for all three viruses ('Period 2'). We used GraphPad Prism version 9.4.1 (GraphPad Software, San Diego, USA) for the analyses, and the 95%CI calculations used the default hybrid Wilson-Brown method. Positive predictive values and negative predictive values including 95%CIs were calculated for a range of hypothetical prevalence rates, using MedCalc (MedCalc Software Ltd. Diagnostic test evaluation calculator. https://www.medcalc.org/calc/diagnostic_test.php (Version 22.007)).

Results

Between the 9th of March 2022 and the 8th of March 2023, 7886 symptomatic patients were tested using FebriDx in the Emergency Department. Of these patients 7849 patients had a valid FebriDx result recorded, and 5586 patients had both a valid FebriDx and RT-PCR results. 160 patients had influenza A or B, SARS-CoV-2, or RSV detected with a Ct value ≥ 35 (reported as low-level RNA detected) and were excluded. Therefore, 5426 patients were included in this analysis. Figure 1 shows the study profile.

Over the 12 months of the study, 999 (18.4%) of 5426 patients had influenza A or B detected, 520 (9.6%) patients had SARS-CoV-2, and 190 (3.5%) had RSV. Of the 999 patients who had influenza detected, 963 (96.4%) had influenza A detected and 36 (3.6%) had influenza B detected. There were 34 co-infections with two viruses, and no co-infection with more than two viruses, and no patients had both influenza A and B co-detected.

Patients had a median age of 62 years [IQR 36–78]. 2842 of the 5426 patients (52.4%) were female. 74.6% of patients were White British with a wide range of other ethnicities represented at low frequencies. Patients had a median indices of multiple deprivation (IMD) decile of 5 (1 being the most deprived and 10 being the least). Comorbidities were common, with 45.0% of patients having cardiovascular disease, 45.9% having respiratory disease, 37.8% having renal disease, and 29.8% having obesity. 64.2% of patients were admitted to an inpatient ward from the Emergency Department, with a median length of stay for those admitted of 3 days [IQR 1–8]. In hospital mortality was low at 2.8%. 2725 of 5426 patients (50.2%) had positive FebriDx MxA test results and 2701 (49.8%) had negative FebriDx MxA results. Baseline characteristics and outcomes are shown in Table 1.

There were differences in baseline characteristics and clinical outcomes between patients with different respiratory virus infections. There was a difference in age across the groups with patients with influenza being younger adults with a median age of 36 [25–61], patients with SARS-CoV-2 had a median age of 63 [36–81], and patients with RSV had a median age of 68 [42–83] ($p<0.0001$). There was a difference in sex across the groups with a high proportion of patients with influenza (55.7%) and RSV (58.9%) being female compared to those with SARS-CoV-2 (48.7%), ($p=0.011$). There were also differences in ethnicity, with 63.1% of influenza patients being White British, compared to 70.0% of SARS-CoV-2 patients, and 73.2%

of RSV patients (p=0.003) although many patients were of unknown ethnicity (10.4% overall). Patients with influenza and SARS-CoV-2 had a lower median IMD decile (i.e. were from more deprived areas) compared to patients with RSV. 59.7% of influenza patients had at least one comorbidity, 76.3% of patients with SARS-CoV-2 had at least one comorbidity, and 85.3% of patients with RSV had at least one comorbidity (p<0.0001). Only 28.4% of patients with influenza were admitted to hospital from the ED, compared to 43.8% of patients with SARS-CoV-2, and 58.4% of patients with RSV (p<0.0001). Of those admitted, median length of hospital stay was 2 days [0–6] for patients with influenza, 5 days [2–10] for patients with SARS-CoV-2, and 3 days [0–6] for patients with RSV (p<0.0001). There were no statistical differences for in-hospital mortality.

The overall sensitivity of FebriDx MxA compared to RT-PCR was 93.3% (95%CI 91.6–94.7) for influenza, 85.0% (81.7–87.8) for SARS-CoV-2, 73.2% (66.4–79.0) for RSV, and 88.4% (86.8–89.9) for all three viruses combined (p<0.0001). The specificity was 59.5% (58.0–60.9) for influenza, 53.5% (52.1–54.9) for SARS-CoV-2, 50.6% (49.3–52.0) for RSV, and 66.8% (65.3–68.3) for all three viruses combined.

The NPV was 97.5% (96.9–98.0) for influenza, 97.1% (96.4–97.7) for SARS-CoV-2, 98.1% (97.5–98.6) for RSV, and 92.8% (91.8–93.7) for all three viruses combined. Table 2 shows the diagnostic accuracy measures for individual viruses and all three combined.

The frequency of detection of the different respiratory viruses fluctuated significantly over time in the point-of-care testing service (Figure 2). There were several periods of increased detection of SARS-CoV-2 during which detections of influenza and RSV were minimal. Influenza had a single notable peak in detections in December 2022 to January 2023, with RSV also being more frequently detected in this timeframe.

Diagnostic accuracy was therefore also calculated for two eight-weeks peak periods of differing respiratory virus activity, as annotated in Figure 2 and described in Table 3. Period 1 represents a period of SARS-CoV-2 detection with almost no co-circulation of influenza and RSV, and was from 09/06/2022 to 04/08/2022 with 620 patients tested in this period. During period 1, the prevalence of SARS-CoV-2 was 10.5% (8.3–13.1), the sensitivity of FebriDx MxA for SARS-CoV-2 was 87.7% (77.6–93.6), and the NPV was 97.9% (95.9–98.9). Period 2 represents a period of high co-circulation of all three viruses and was from 20/11/2022 to

15/01/2023 with 1607 patients tested. During period 2, the prevalence of influenza was very high at 45.1% (42.6–47.5%), the sensitivity of FebriDx MxA for influenza was 93.6% (91.6–95.2) and the NPV was 91.4% (88.8–93.5). For SARS-CoV-2, the prevalence was 7.7% (6.5–9.1), the sensitivity of FebriDx MxA was 84.6 (77.1–89.9), and the NPV was 96.5% (94.5–97.7). For RSV, the prevalence was 7.3% (6.1–8.7), the sensitivity of FebriDx MxA was 74.4% (65.8–81.4) and the NPV was 94.4% (92.1–96.1).

NPVs were also calculated for a range of hypothetical prevalence rates (Table 4). High NPVs were generally preserved at range of prevalence rates for influenza, SARS-CoV-2, RSV and all three viruses combined.

Discussion

In this large, real-world study of symptomatic adult patients in the Emergency Department, we have shown that FebriDx MxA has a high NPV for the detection of influenza, SARS-CoV-2, and RSV individually, and for all three viruses combined, compared to RT-PCR. FebriDx MxA NPV for all three viruses individually and combined were preserved during a period of high SARS-CoV-2 activity alone, and when all three viruses were co-circulating and at a wide range of prevalence rates for all three viruses. These finding indicate that FebriDx MxA continues to be a useful triage tool in symptomatic patients in the Emergency Department in the context of the return of respiratory virus co-circulation and widespread COVID-19 vaccination, and remains superior to clinical triage.

The high sensitivity and NPV of FebriDx MxA for SARS-CoV-2 in this study are comparable to other studies of FebriDx MxA used in the Emergency Department in symptomatic adults during the first and second wave of the pandemic in the UK and internationally, before vaccination was available and when co-circulation of other respiratory viruses was limited.[2–10]

The high NPV of FebriDx MxA test for all three respiratory viruses is important for the prevention of hospital-acquired respiratory virus infection as the early ruling out of influenza or RSV in addition to COVID-19 allows EDs to separate infected and non-infected patients and

to maintain patient flow and operational capacity. Prevention of nosocomial acquisition and outbreaks of COVID-19 in hospital has received significant attention, however, hospital-acquired influenza and RSV infections are likely under-reported and are also associated with high morbidity and mortality.[20,21] Point-of-care testing strategies have been shown to reduce hospital-acquired COVID-19 and the use of the FebriDx as a triage tool in ED would be likely to similarly reduce nosocomial influenza and RSV infection but may represent a more rapid and cost effective solution compared to PCR .[22]

To our knowledge, this is the largest published study evaluating the performance of FebriDx with over five thousand patients, and the first study to evaluate the diagnostic accuracy for influenza and RSV, in adults in the Emergency Department. Additionally, it is the first study to report on FebriDx MxA performance in patients infected with SARS-CoV-2 attending an Emergency Department after the introduction of widespread COVID-19 vaccination, with the exception of one small study of fewer than 100 patients.[23]

FebriDx MxA point-of-care testing demonstrated high sensitivities and NPVs for a range of important respiratory viruses with outbreak, and in the case of influenza, pandemic potential. The high NPV was preserved across varying prevalence and co-circulation rates. This data suggests that point-of-care MxA testing could be used as a triage tool in future novel viral pandemics including those caused by novel coronaviruses and avian influenza. Furthermore, as MxA is an antiviral host response that is induced by a broad range of viruses, rather than a test targeting a specific viral antigen or RNA sequence, it could be used early in a viral pandemic before specific rapid molecular diagnostic tests are widely available and would be highly likely to be superior to clinical triage.

The sensitivity of FebriDx MxA was highest in influenza positive patients compared to the other viruses. The reasons behind this are not clear but potentially include stronger or prolonged induction of MxA compared to other viruses. In addition, as we were not able to collect data on the duration of illness prior to ED presentation in this study, it is possible that patients with Influenza presented earlier in the course of their illness, when MxA levels were higher compared to the other viruses when RNA is still detectable but MxA levels have waned. There were also important differences in baseline characteristic of influenza positive patients compared to the other viruses including a dramatically lower median age, a higher proportion of ethnically Asian patients, and lower levels of comorbidity. In addition, patients with

influenza were much less frequently admitted to hospital and when they were admitted had a lower length of stay than patients with SARS-COV-2 or RSV. The reasons behind these differences cannot be directly inferred from this study but they could represent a change in health seeking behaviour in younger adults following the pandemic, with large numbers of otherwise healthy, younger patient presenting to ED, being diagnosed with influenza, and being subsequently discharged home, rather than presenting to primary care or not seeking medical attention at all.

The sensitivity of the FebriDx MxA for RSV was notably lower than for influenza and SARS-CoV-2 patients. Patients with RSV were older, had more comorbidities, and were more frequently admitted to hospital than patients with influenza or SARS-CoV-2. A possible explanation for the lower sensitivity of MxA in RSV is that increased age, frailty, and immunosenescence were associated with an impaired immune antiviral response compared to the other viruses. Alternatively, patients with RSV may be presenting later in the course of their illness with secondary bacterial infection when the antiviral host response has waned but viral RNA remains detectable. As we were not able to reliably collect data on duration of illness or markers of bacterial infection in this cohort further studies are needed to further evaluate this interesting finding.

The large cohorts of patients described in this study offer important insights into the differences in characteristics and clinical outcomes between patients with different respiratory virus infections, which warrants further investigation in large prospective studies.

The specificity of FebriDx for all three viruses combined (66.8%) was lower than in previous studies (typically >90%).[2–7] This lower specificity is likely due to the return of other respiratory viruses that were not circulating at high levels during the first waves of the COVID-19 pandemic when these previous studies took place and are not tested for by the RT-PCR test that we used, such as rhinovirus, human metapneumovirus, parainfluenza and adenovirus.[8–9] Additionally, infection with non-respiratory viruses might also be contributing to the detection of antiviral host response in these patients. This study highlights the issue of testing for a small number of respiratory viruses by near-patient RT-PCR when syndromic molecular point-of-care testing for a wide-range of respiratory viruses may provide additional benefits in patient care.[24–27]

The strengths of this study include the high number of patients, the duration of the study over a full year including variations in respiratory virus prevalence, and the broad inclusion criteria and limited exclusion criteria. The high rate of comorbidities was broadly similar to patient characteristics in previous studies of patients presenting to hospital with acute respiratory illness.[24,25] The FebriDx has recently received US FDA approval.[28] These factors, plus the setting of a typical large Emergency Department, suggest that this study's findings are likely to be highly useful and highly generalisable to other centres nationally and internationally.

The limitations of this study include the exclusion of immunosuppressed patients, children, and asymptomatic patients, and the single-centre setting. Whilst individual patient data for vaccination was not available, the COVID-19 vaccination rate locally in Southampton is high, with over 80% of people aged over 75 years receiving an Autumn 2022 booster.[29]

In conclusion, this study shows that FebriDx MxA has a high NPV for influenza and RSV, and retains a high NPV for SARS-CoV-2, in the context of virus co-circulation and widespread COVID-19 vaccination. FebriDx MxA continues to be valuable as a 'rule out' triage tool in patients with acute respiratory illness in the Emergency Department and could be scaled to provide a national triage tool in future viral pandemics.

Funding

There was no specific funding for this study. The FebriDx and RT-PCR tests were purchased from a UK distributor by University Hospital Southampton NHS Foundation Trust as part of clinical service. The manufacturers of FebriDx (Lumos Diagnostics) had no input into the conception, design, or conduct of this study or the writing of the manuscript.

Author contributions

NJB conceived of and designed the study, reviewed the medical literature, analysed and interpreted the data, and wrote the manuscript. CD designed the study, and extracted and analysed the data. TWC conceived of and designed the study, reviewed the medical literature, and analysed and interpreted the data, and supervised the manuscript development. TWC, NW, and MC oversaw the RT-PCR point-of-care testing service. TWC, NJB, DW, CH, and MC oversaw the FebriDx testing service. All authors contributed to and have reviewed the manuscript.

Declaration of Competing Interest

TWC has received equipment and consumables at discount or free of charge for the purposes independent of research, outside of this submitted study, from BioFire diagnostics, BioMerieux, QIAGEN and SenseBio. He has received travel re-imbursement and speaker fees from BioFire diagnostics, BioMerieux, and QIAGEN. He has received consultancy fees from Cepheid, Synairgen research, Roche, Sanofi, BioMerieux and Biofire diagnostics. He has received honoraria for participation in advisory boards from Cepheid, Roche, Janssen, GSK, Shionogi, Seqirus and Sanofi. He is a member of an independent data monitoring committee for a trial sponsored by Roche. He has acted as the UK chief investigator for a study sponsored by Janssen. He owns shares in Synairgen research. All other authors declare no competing interests.

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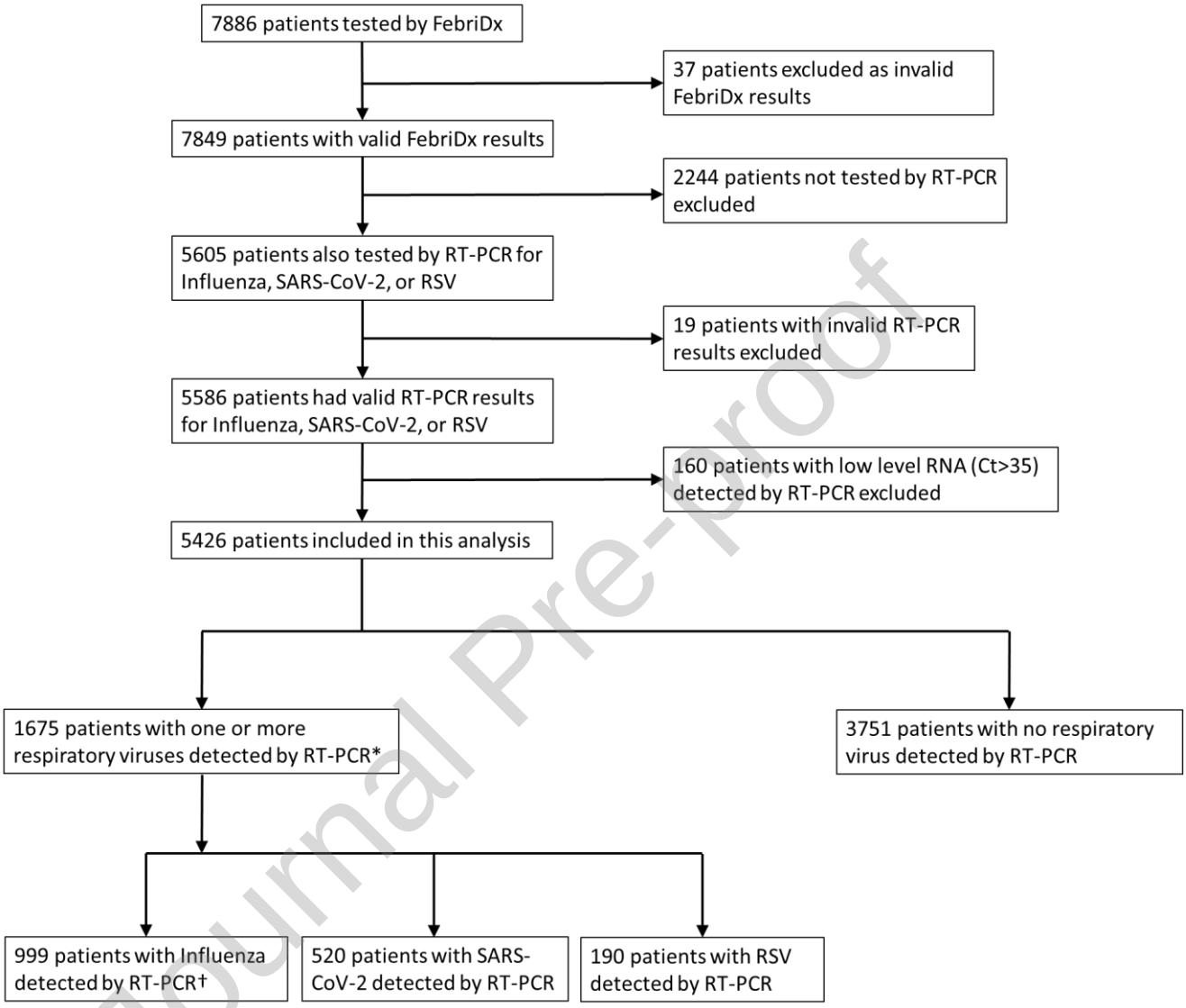
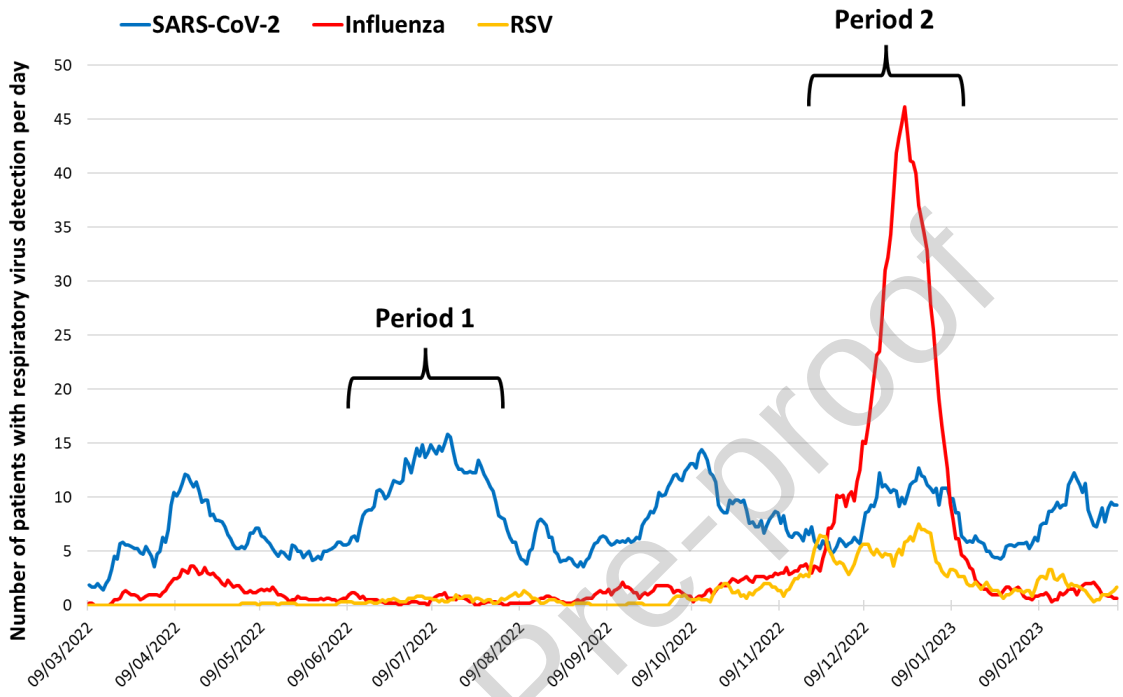
Figures

Figure 1: Study profile



RSV=Respiratory Syncytial Virus. *There were 34 co-detections. †There were 963 patients with influenza A detected and 36 patients with influenza B detected.

Figure 2: Detection of respiratory viruses over time by point-of-care RT-PCR testing in the Acute Medicine Unit, March 2022–2023



Number of patients with respiratory virus detection per day by RT-PCR is displayed as a seven-day rolling mean average. Period 1 is an eight-week period of high SARS-CoV-2 circulation with very low co-circulation of other respiratory viruses. Period 2 is an eight-week period of co-circulation for all three viruses.

Tables

Table 1: Characteristics of symptomatic patients in the ED tested by FebriDx and RT-PCR

	All patients (n=5426)	Influenza patients (n=999)	SARS-CoV-2 patients (n=520)	RSV patients (n=190)	p value*
Demographics					
Age, years	62 [36–78]	36 [25–61]	63 [36–81]	68 [42–83]	<0.0001
Age ≥ 65 years	2511 (46.3%)	227 (22.7%)	252 (48.5%)	103 (54.2%)	<0.0001
Female sex	2842 (52.4%)	556 (55.7%)	253 (48.7%)	112 (58.9%)	0.011
IMD decile†	5 [3–8]	5 [3–7]	5 [3–8]	6 [3–8]	0.011
Ethnicity					
White British	4047 (74.6%)	630 (63.1%)	364 (70.0%)	139 (73.2%)	0.003
White other/Irish	269 (5.0%)	69 (6.9%)	34 (6.5%)	8 (4.2%)	..
Indian, Pakistani, Bangladeshi, & Other Asian	251 (4.6%)	80 (8.0%)	26 (5.0%)	7 (3.7%)	..
Chinese	61 (1.1%)	17 (1.7%)	18 (3.5%)	0 (0%)	..
Black including African & Caribbean	60 (1.1%)	13 (1.3%)	9 (1.7%)	0 (0%)	..
Mixed	54 (1.0%)	14 (1.4%)	3 (0.6%)	2 (1.1%)	..
Other	119 (2.2%)	39 (3.9%)	12 (2.3%)	6 (3.2%)	..
Unknown	565 (10.4%)	137 (13.7%)	54 (10.4%)	28 (14.7%)	..
Comorbidities					
Any comorbidity	4372 (80.6%)	596 (59.7%)	397 (76.3%)	162 (85.3%)	<0.0001
Obesity	1616 (29.8%)	204 (20.4%)	130 (25.0%)	54 (28.4%)	..
Diabetes Mellitus	1064 (19.6%)	125 (12.5%)	104 (20.0%)	34 (17.9%)	..
Hypertension	2146 (39.6%)	192 (19.2%)	203 (39.0%)	78 (41.1%)	..
Cardiovascular disease	2441 (45.0%)	208 (20.8%)	236 (45.4%)	87 (45.8%)	..
Respiratory disease	2489 (45.9%)	299 (29.9%)	204 (39.2%)	96 (50.5%)	..
Renal disease	2051 (37.8%)	166 (16.6%)	179 (34.4%)	72 (37.9%)	..
Liver disease	278 (5.1%)	16 (1.6%)	23 (4.4%)	9 (4.7%)	..
Cancer	1178 (21.7%)	106 (10.6%)	91 (17.5%)	41 (21.6%)	..

Immunosuppressed	11 (0.2%)	1 (0.1%)	1 (0.2%)	1 (0.5%)	..
Dementia	291 (5.4%)	17 (1.7%)	38 (7.3%)	11 (5.8%)	..
Clinical outcomes					
Admitted to hospital	3484 (64.2%)	284 (28.4%)	228 (43.8%)	111 (58.4%)	<0.0001
Length of hospital stay, days	3 [1–8]	2 [0–6]	5 [2–10]	3 [0–6]	<0.0001
In-hospital mortality	98/3484 (2.8%)	4/284 (1.4%)	9/228 (3.9%)	5/111 (4.5%)	0.125
FebriDx MxA Result					
Detected	2725 (50.2%)	932 (93.3%)	442 (85.0%)	139 (73.2%)	<0.0001
Not detected	2701 (49.8%)	67 (6.7%)	78 (15.0%)	51 (26.8%)	..

Data are n (%) or median [IQR]. RSV=Respiratory Syncytial Virus. IMD=Indices of multiple deprivation. *Comparison between respiratory virus patient groups. †Data available for 5382 patients.

Table 2: Diagnostic accuracy measures of FebriDx MxA for Influenza, SARS-CoV-2, RSV, and all three viruses combined compared to RT-PCR in symptomatic patients in the ED (n=5426)

	n/N	Percentage	95%CI
Influenza			
Sensitivity	932/999	93.3	91.6 – 94.7
Specificity	2634/4427	59.5	58.0 – 60.9
PPV	932/2725	34.2	32.4 – 36.0
NPV	**2634/2701**	**97.5**	**96.9 – 98.0**
Prevalence	999/5426	18.4	17.4 – 19.5
SARS-CoV-2			
Sensitivity	442/520	85.0	81.7 – 87.8
Specificity	2623/4906	53.5	52.1 – 54.9
PPV	442/2725	16.2	14.9 – 17.7
NPV	**2623/2701**	**97.1**	**96.4 – 97.7**
Prevalence	520/5426	9.6	8.8 – 10.4
RSV			
Sensitivity	139/190	73.2	66.4 – 79.0
Specificity	2650/5236	50.6	49.3 – 52.0

PPV	139/2725	5.1	4.3 – 6.0
NPV	**2650/2701**	**98.1**	**97.5 – 98.6**
Prevalence	190/5426	3.5	3.0 – 4.0
All three viruses combined			
Sensitivity	1481/1675	88.4	86.8 – 89.9
Specificity	2507/3751	66.8	65.3 – 68.3
PPV	1481/2725	54.3	52.5 – 56.2
NPV	**2507/2701**	**92.8**	**91.8 – 93.7**
Prevalence	1675/5426	30.9	29.6 – 32.1

RSV=Respiratory Syncytial Virus, PPV=positive predictive value, NPV=negative predictive value.

Table 3: Diagnostic accuracy measures of FebriDx MxA during periods of high circulation for SARS-CoV-2 alone (Period 1) and co-circulation of all three viruses (Period 2)

	n/N	**Percentage**	**95%CI**
Period 1: High SARS-CoV-2 circulation alone			
Influenza			
Sensitivity	16/17	94.1	73.0 – 99.7
Specificity	379/603	62.9	58.9 – 66.6
PPV	16/240	6.7	4.1 – 10.6
NPV	**379/380**	**99.7**	**98.5 – 100.0**
Prevalence	17/620	2.7	1.7 – 4.3
SARS-CoV-2			
Sensitivity	57/65	87.7	77.6 – 93.6
Specificity	372/555	67.0	63.0 – 70.8
PPV	57/240	23.8	18.8 – 29.5
NPV	**372/380**	**97.9**	**95.9 – 98.9**
Prevalence	65/620	10.5	8.3 – 13.1
RSV			
Sensitivity	9/12	75.0	46.8 – 91.1
Specificity	377/608	62.0	58.1 – 65.8
PPV	9/240	3.8	2.0 – 7.0
NPV	**377/380**	**99.2**	**97.7 – 99.8**
Prevalence	12/620	1.9	1.1 – 3.4
All three viruses combined			
Sensitivity	81/93	87.1	78.8 – 92.5
Specificity	368/527	69.8	65.8 – 73.6
PPV	81/240	33.8	28.1 – 40.0
NPV	**368/380**	**96.8**	**94.6 – 98.2**

Prevalence	93/620	15.0	12.4 – 18.0
Period 2: All three viruses co-circulating			
Influenza			
Sensitivity	678/724	93.6	91.6 – 95.2
Specificity	491/883	55.6	52.3 – 58.9
PPV	678/1070	63.4	60.4 – 66.2
NPV	**491/537**	**91.4**	**88.8 – 93.5**
Prevalence	724/1607	45.1	42.6 – 47.5
SARS-CoV-2			
Sensitivity	104/123	84.6	77.1 – 89.9
Specificity	518/1484	34.9	32.5 – 37.4
PPV	104/1070	9.7	8.1 – 11.6
NPV	**518/537**	**96.5**	**94.5 – 97.7**
Prevalence	123/1607	7.7	6.5 – 9.1
RSV			
Sensitivity	87/117	74.4	65.8 – 81.4
Specificity	507/1490	34.0	31.7 – 36.5
PPV	87/1070	8.1	6.6 – 9.9
NPV	**507/537**	**94.4**	**92.1 – 96.1**
Prevalence	117/1607	7.3	6.1 – 8.7
All three viruses combined			
Sensitivity	844/938	90.0	87.9 – 91.7
Specificity	443/669	66.2	62.6 – 69.7
PPV	844/1070	78.9	76.3 – 81.2
NPV	**443/537**	**82.5**	**79.1 – 85.5**
Prevalence	938/1607	58.4	55.9 – 60.8

RSV=Respiratory Syncytial Virus, PPV=positive predictive value, NPV=negative predictive value. Period 1 was from 09/06/2022 to 04/08/2022. During Period 1 there were 17 patients with influenza A detected and no influenza B detections. Period 2 was from 20/11/2022 to 15/01/2023. During Period 2 there were 714 patients with influenza A detected and 10 with influenza B detected.

Table 4: Negative predictive values of FebriDx MxA over a range of hypothetical prevalence rates for Influenza, SARS-CoV-2, RSV, and all three viruses combined

Prevalence	**NPV (%)**	**95% CI (%)**
Influenza		
0.10%	100.0	99.8 – 100.0
1%	99.9	99.7 – 100.0
5%	99.4	99.0 – 99.7
10%	98.8	98.3 – 99.1
20%	97.3	96.6 – 97.8
50%	89.9	88.7 – 91.0

SARS-CoV-2		
0.10%	100.0	99.8 – 100.0
1%	99.7	99.4 – 99.9
5%	98.5	98.0 – 99.0
10%	97.0	96.3 – 97.6
20%	93.5	92.5 – 94.4
50%	78.1	76.5 – 79.7
RSV		
0.10%	100.0	99.8 – 100.0
1%	99.5	99.1 – 99.7
5%	97.3	96.6 – 97.9
10%	94.4	93.5 – 95.3
20%	88.3	87.0 – 89.5
50%	65.3	63.5 – 67.1
All three viruses combined		
0.10%	100.0	99.8 – 100.0
1%	99.8	99.6 – 100.0
5%	99.1	98.7 – 99.4
10%	98.1	97.5 – 98.6
20%	95.9	95.0 – 96.6
50%	85.2	83.8 – 86.6

RSV=Respiratory Syncytial Virus, NPV=negative predictive value.

Declaration of Competing Interest

TWC has received equipment and consumables at discount or free of charge for the purposes independent of research, outside of this submitted study, from BioFire diagnostics, BioMerieux, QIAGEN and SenseBio. He has received travel re-imbursement and speaker fees from BioFire diagnostics, BioMerieux, and QIAGEN. He has received consultancy fees from Cepheid, Synairgen research, Roche, Sanofi, BioMerieux and Biofire diagnostics. He has received honoraria for participation in advisory boards from Cepheid, Roche, Janssen, GSK, Shionogi, Seqirus and Sanofi. He is a member of an independent data monitoring committee for a trial sponsored by Roche. He has acted as the UK chief investigator for a study sponsored by Janssen. He owns shares in Synairgen research. All other authors declare no competing interests.

Highlights

- Largest study evaluating diagnostic accuracy of FebriDx MxA for respiratory viruses

- First large study of FebriDx MxA during virus co-circulation and after COVID-19 vaccine introduction
- The Negative Predictive Value (NPV) was 97.5% for influenza, 97.1% for SARS-CoV-2, 98.1% for RSV, & 92.8% for all 3 viruses combined
- High NPVs retained during different prevalence rates of respiratory virus
- FebriDx is a useful 'rule out' tool in ED and a potential triage tool in a future pandemic